PART I - ADMINISTRATIVE

Section 1. General administrative information

Title of project	
Enhancement of salmonid gamete quality by manipulation of intracellular ATP	
BPA project number	**20047**
Contract renewal date (mm/yyyy)	
Multiple actions? (indicate Yes or No)	
Business name of agency, institution or organization requesting funding	
University of Idaho	
Business acronym (if appropriate)	
Proposal contact person or principal investigator:	
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NPPC Program Measure Number(s) which this project addresses	
1994 Columbia River Basin Fish and Wildlife Program: 7.2, 7.4D, 7.4E	
FWS/NMFS Biological Opinion Number(s) which this project addresses	
Other planning document references	
Short description	
Develop methods to enhance the short-term storage of unfrozen salmonid gametes and improve the quality of salmonid eggs and nonactivated, activated, and cryopreserved sperm.	
Target species	
Oncorhynchus tshawytscha (chinook) and Oncorhynchus mykiss (steelhead/rainbow)	

Section 2. Sorting and evaluation

Subbasin
Systemwide

Evaluation Process Sort

CBFWA caucus	CBFWA eval. process	ISRP project type
X one or more caucus	If your project fits either of	X one or more categories

		these processes, X one or both			
x	Anadromous fish	x	Multi-year (milestone-based evaluation)		Watershed councils/model watersheds
	Resident Fish		Watershed project eval.		Information dissemination
	Wildlife				Operation & maintenance
					New construction
				x	Research & monitoring
					Implementation & mgmt
					Wildlife habitat acquisitions

Section 3. Relationships to other Bonneville projects

Umbrella / sub-proposal relationships. List umbrella project first.

Project #	Project title/description

Other dependent or critically-related projects

Project #	Project title/description	Nature of relationship
	Intracytoplasmic Sperm Injection: Genetic Retrieval from Single Sperm (Dr. Joseph G. Cloud)	Project Participant
	Endocrine Control of Ovarian Development in Salmonids (Dr. James J. Nagler)	Project Participant
	Analyzing Genetic and Behavioral Changes During Salmonid Domestication (Dr. Gary Thorgaard et al.)	Project Participant
	Induction of Precocious Sexual Maturity and Enhanced Egg Production in Fish (Dr. Gerald Schelling)	Project Participant

	Viral Vaccines and Effects on Reproductive Status (Dr. Sandra Ristow)	Project Participant

Section 4. Objectives, tasks and schedules

Past accomplishments

Year	Accomplishment	Met biological objectives?

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Evaluate whether a correlation exits between sperm ATP (adenosine triphosphate) content and sperm motility and fertilizing capability	a	Correlate ATP content and sperm motility and fertility for sperm from different males
		b	Correlate phosphocreatine (PCr) content and sperm motility and fertility
2	Evaluate whether sperm ATP and/or PCr content can be increased by manipulations of short-term storage conditions	a	Examine whether sperm, depleted of ATP by transient exposure to anoxia, can regenerate ATP stores by short-term maintenance with nutrients and 100% oxygen
		b	Examine whether such maintenance conditions will lead to elevated ATP stores in untreated as well as activated sperm
3	Evaluate the effects of cryopreservation on the relationship between ATP and/or PCr content and sperm motility and fertility	a	Examine whether a positive relationship persists between ATP and/or PCr content and motility and fertility of cryopreserved sperm

Objective schedules and costs

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1	10/1999	10/2000	establish ATP:motility & ATP:fertility correlations		33.3
2	10/1999	10/2000	establish whether ATP content can be increased		33.3
3	10/1999	10/2000	establish whether ATP content correlates with motility/fertility of cryopreserved sperm		33.3
				Total	100

Schedule constraints
None
Completion date
10/2004

Section 5. Budget

FY99 project budget (BPA obligated):	$**0**

FY2000 budget by line item

Item	Note	% of total	FY2000 ($)
Personnel	Rolf Ingermann (3.0 mo); Joseph Cloud (1.0 mo), (James Nagler after FY2000), 1 Technician (12.0 mo), 1 Graduate Student (12.0 mo), 0.5 Undergraduate Student (750 hr)	41.5	76,000
Fringe benefits	RLI, JGC, Tech: 28.5%, GS: 1%, UGS: 1% during school year, 9% during summer	9.0	16,410
Supplies, materials, non-expendable property	Supplies (7,000), NMR & Flow Cytometer use (6,000)	7.1	13,000
Operations & maintenance	Graduate Student Tuition & Fees (2,500), Vehicle Rental (1,000), Publication Costs, Xeroxing, Graphics (1,000), Equipment Maintenance (3,500)	4.4	8,000
Capital acquisitions or	Equipment: Microcentrifuge (2,500),	2.7	5,000

improvements (e.g. land, buildings, major equip.)	Laboratory Computer (2,500)		
NEPA costs		0	0
Construction-related support		0	0
PIT tags	# of tags:	0	0
Travel		1.6	3,000
Indirect costs	44.7%	30.9	56,505
Subcontractor		0	0
Other	Center for Reproductive Biology Administrative Core (5,000)	2.7	5,000
TOTAL BPA REQUESTED BUDGET			182,915

Cost sharing

Organization	Item or service provided	% total project cost (incl. BPA)	Amount ($)
Total project cost (including BPA portion)			

Outyear costs

	FY2001	FY02	FY03	FY04
Total budget	193,761	203,450	213,622	224,304

Section 6. References (No Watershed References)

Watershed	Reference
	Ballard WW. 1973. Normal embryonic stages for salmonid fishes, based on *Salmo gairdneri* Richardson and *Salvelinus fontinalis* (Mitchill). J Exp Zool, 184:7-26.
	Benau J, Terner C. 1980. Initiation, prolongation and reactivation of the motility of salmonid spermatozoa. Gamete Res, 3:247-257.
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	Comhaire F, Vermeulen L, Schoonjans F. 1987. Reassessment of the accuracy of traditional sperm characteristics and adenosine triphosphate (ATP) in estimating the fertilizing potential of human semen *in vivo*. Intl J Androl, 10:653-662.
	Du Toit D, Bornman MS, Van Der Merwe MP, Du Plessis DJ, Oosthuizen JM. 1993. Differential sperm motility scoring and sperm ATP concentrations. Arch Androl, 30:69-71.
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	Lahnsteiner F, Patzner RA, Weismann T. 1993. Energy resources of spermatozoa of the rainbow trout *Oncorhynchus mykiss* (Pisces, Teleostei). Reprod Nutr Dev, 33:349-360.
	McLaughlin EA, Ford WCL, Hull MGR. 1994. Adenosine triphosphate and motility characteristics of fresh and cryopreserved human spermatozoa. Intl J Androl, 17: 19-23.
	Moccia RD, Munkittrick KR. 1987. Relationship between fertilization of rainbow trout *(Salmo gairdneri*) eggs and the motility of spermatozoa. Theriogenology, 27: 679-688.
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PART II - NARRATIVE

Section 7. Abstract

Accurate prediction (and enhancement) of fish sperm and egg quality and fertilizing capability is very important in maximizing the fertility and the success of artificial reproduction, particularly with gametes from rare and endangered salmonid species. There are currently few reliable predictors of such characteristics. Our preliminary data suggest that the adenosine triphosphate (ATP) content of the sperm and egg is such a reliable predictor. (ATP is the universal currency of energy within cells.) Another candidate is the phosphocreatine (PCr) content as PCr can react with adenosine diphosphate to quickly generate additional ATP. Salmonid sperm are motile for less than a minute and appear to rely on stored ATP (and possibly PCr) for successful motility and fertility. Further, it is likely that the amount of stored ATP (and/or PCr) exerts an influence on energy-dependent events which occur rapidly in the egg at fertilization. Consequently, we **hypothesize that there is a direct relationship between ATP, and possibly PCr, content and motility and fertilizing ability of salmonid sperm and eggs**. We will expand the hypothesis to test relationship between ATP/PCr and sperm motility/fertility with cryopreserved as well as fresh samples. We predict that ATP content of frozen/thawed sperm will correlate positively with

sperm function. If our hypothesis proves to be correct as our preliminary data suggest, our attention will be directed to establishing ways in which ATP (and possibly PCr) content can be maintained or even increased to enhance fertilizing success of precious samples of gametes. The results of these proposed 5-year studies should increase our basic understanding of gamete physiology of salmonids and thus be of fundamental significance. The results of this study should also support the development of methods which extend, and potentially increase, and thus enhance the fertilizing capabilities of precious samples. For example, it may be possible to increase fertilizing capability of sperm and/or eggs from poor adults, of gametes following shipment, or of cryopreserved sperm. Such a capability could be particularly valuable in increasing the chances for success in salmonid recovery programs. The results of these studies will be shared with hatchery personnel and published in peer-reviewed, scientific journals with global distributions.

Section 8. Project description

a. Technical and/or scientific background

Current methodologies in artificial fish reproduction and hatchery production are highly successful. Furthermore, with concerns about inbreeding and loss of variability in hatchery propagation, it appears unnecessary and perhaps even undesirable to attempt to increase artificial fish reproductive success of standard brood stocks. However, mechanisms to improve reproductive success of rare and endangered salmonid species is highly desirable.

Currently, there are few reliable indicators of sperm quality or the extent of damage related to sperm preparation and storage. Also, there are no reliable methods to increase sperm quality. One evaluator of semen quality is sperm motility (Du Toit et al., 1993) and in fish, motility and fertilizing ability are highly correlated (Stoss, 1983, Ciereszko & Dabrowski, 1994). Unfortunately, fish sperm are motile for such a short period of time that this assessment has significant limitations. (For example, in trout, sperm motility lasts less than one minute; Lahnsteiner et al., 1993.) With the exception of several enzyme activities (Ciereszko & Dabrowski, 1994), no other parameter has proven to be particularly useful in assessing semen quality. A likely candidate, however, is the adenosine triphosphate (ATP) content of sperm. Also, ATP can be generated quickly from phosphocreatine (PCr) in at least several types of tissue by the reaction PCr + ADP $\leftrightarrow$ Cr + ATP (catalyzed by creatine kinase). Therefore, PCr becomes a reasonable candidate as well.

ATP is the primary energy source used by the sperm flagellum to generate propulsion (Redondo-Muller et al., 1991; Bhattacharyya & Pakrashi, 1993). Consequently, mammalian sperm ATP content and motility have received recent attention although there is no consensus as to their relationship (Comhaire et al., 1983, 1987; Calamera et al., 1982; Fourie et al., 1991; Gottlieb et al., 1991; Vigue et al., 1992; Du Toit et al., 1993; McLaughlin et al., 1994). Relative to mammalian sperm, fish sperm have fewer mitochondria and shorter durations of motility suggesting a greater reliance on stored ATP (and possibly PCr) for motility and fertility. Indeed, prior to motility trout sperm retain high levels of ATP and PCr, initiation of motility results in a rapid decrease of both, and a decrease in motility correlates with the depletion of ATP and possibly, PCr (Christen et al.,1987; Robitaille et al., 1987). (Similar findings have been reported for carp sperm [Perchec et al., 1995].) The decrease in ATP with motility appears to be due to an inability of oxidative phosphorylation to maintain ATP synthesis at rates commensurate with ATP breakdown (Benau & Terner, 1980; Christen et al., 1987). Results from our laboratory indicate

positive correlation exists between salmonid sperm ATP content and percent of motile sperm (Bencic et al., In Press). Whether this relationship holds for cryopreserved sperm is not known but will be examined in this proposed project.

Salmonid sperm shed into natural (hypotonic) waters lyse rapidly enough that limitations in ATP content (and the oxidative capacity to synthesize more) are of minor consequence (Christen et al., 1987). However, artificial fertilization occurs by mixing gametes in isotonic media. Under such conditions, trout sperm can metabolize endogenous fatty acids to generate ATP *AFTER* motility has ended (Lahnsteiner et al., 1993). *Interestingly, motility could be reinitiated in these sperm*. Clearly, motility in these sperm does not result in irreversible changes which prevent subsequent motility. Therefore, precious sperm used to fertilize eggs may be collected after motility, allowed to regenerate intracellular ATP (and possibly PCr), and used again. Development of maintenance and culture methods which increase intracellular ATP (and possibly PCr) therefore appears particularly significant in maximally utilizing semen samples of limited availability and size.

Brooks et al. (1997) have indicated that factors affecting egg quality are determined by the intrinsic properties of the egg itself and the environment in which the egg is fertilized and incubated. Egg quality is highly variable and the basis for this variability is largely unknown. A potential contributor to this variability is the complement of nutrients deposited within the egg and the ability of the egg to generate ATP which plays the central role in energy exchanges in living systems (Stryer, 1975). Indeed, Boulekbache et al. (1989) have shown with carp eggs that ageing and 'over-ripening' is associated with a decline in ATP content and a decline in the ability of the egg to become fertile. The importance of this ATP decline in the decline of egg quality is not clear. However, Jensen and Alderdice (1984) have shown increased salmonid egg viability at higher air-to-egg ratios suggesting an importance of O_2 and oxidative metabolism, and hence ATP generation, to egg viability maintenance. Overall, the role of ATP (and possibly PCr) in egg quality and fertility is not clear but it seems highly likely that stored ATP has an important role in the egg at fertilization.

Preliminary Studies from Our Laboratory

We have collected data for sperm from both the steelhead trout and chinook salmon, obtained within minutes of adult sacrifice, at Dworshak National Fish Hatchery (Ahsahka, ID). Initial ATP levels have been found to be 12.5 ± 6.4 and 12.1 ± 8.1 pmol/10^6 sperm cells (mean ± SD, N = 18 & 21), respectively. (Seminal fluid alone contains less than 0.1% of the ATP of whole semen; Bencic et al., In Press.) We have found that maintaining semen samples from individual males under different oxygen tensions for 24 hr results in sperm samples with a broad range of intracellular ATP levels and different functional characteristics (Figs. 1 & 2). Whether a relationship exists between PCr and sperm motility and fertility remains to be seen.

We have seen that steelhead and chinook sperm maintained in N_2 for 4 hr show a marked drop (~90%) in ATP. These low ATP levels (and motility) will largely recover when sperm, at least from steelhead trout, are subsequently switched to 100% O_2 (Fig. 3).

We will investigate the effects of cryopreservation on the relationship between sperm ATP and PCr content and sperm motility and fertility. To do so, it is necessary to verify that measured ATP is just intracellular ATP rather than total ATP, including ATP released from damaged cells. We have measured appreciable ectoATPase activity associated with these cells. (EctoATPase activity is ATP hydrolytic activity associated with the exterior of the cell membrane and not due to soluble ATPase activity in the medium from cell damage; Bencic et al., 1997.) We therefore believe that measuring sperm ATP content of thawed semen samples will not be compromised by ATP freed by cell lysis; released ATP is hydrolyzed rapidly by the ectoATPase activity leaving only ATP within intact cells.

We have recently measured the ATP content of individual rainbow trout eggs from 3 females after 24 hr (time involved in shipping the eggs to our laboratory from an out-of-state commercial vendor). Values for eggs from these females were 2.09 ± 0.21, 2.13 ± 0.12, 2.87 ± 0.68, nmol ATP/egg (mean ±SD, *N*=3 for each female). Interestingly, fertility followed the same pattern being highest in those eggs from the female with eggs of the highest ATP content.

b. Rationale and Significance to Regional Programs

Adult returns of wild salmon and steelhead trout in the Pacific Northwest have decreased markedly over the last few decades. It is clear that accurate prediction (and improvement) of fish gamete quality and fertilizing capability is very important in maximizing the success of artificial reproduction, particularly with gametes from rare and endangered salmonid species and populations. These studies seek to elucidate the role of intracellular ATP and PCr in that assessment. If we can establish a positive correlation between intracellular ATP and/or PCr and fertilizing capability of eggs and/or sperm, as our preliminary data suggest (at least for sperm), we

will use the analytical methods of this proposal to examine methods by which intracellular ATP and/or PCr may be maintained or increased in these gametes. We focus our attention on salmonids as there is a wealth of scientific information on these fishes, gametes are available year-round, they have pronounced economic importance, and stocks of these fishes are rare or endangered. Any treatment of gametes from these populations which increases the success rate of artificial fertilization would be highly desirable. For example, it may be possible to increase fertilizing capability of sperm and/or eggs from poor adults, of gametes following shipment, or of cryopreserved sperm. Our experiences in collecting steelhead and chinook gametes from Dworshak National Fish Hatchery have shown that on a given day of spawning, there is often a highly skewed sex ratio of incoming fish. It could be very beneficial to have effective short-term storage protocols to maximize gamete utilization. Such capabilities could be particularly valuable in increasing the chances for success in salmonid recovery programs when numbers of returning fish are low. The proposed studies will also evaluate the effect of cryopreservation on the proposed relationship between ATP (and possibly PCr) content and sperm motility/fertility. Cryopreservation is very important with these species as final maturation between females and males is often out of synchrony (fish of opposite sexes may not even return in the same brood year), it reduces the possibility of losing an entire year's production through hatchery system failure or disease, and it facilitates the shipment of gametes to hatcheries and institutions. Ultimately, the development of mechanisms to evaluate, extend and/or enhance the quality (or the capacity to fertilize) of fresh, stored, and cryopreserved sperm and of eggs from these rare and endangered species is very important.

c. Relationships to other projects

The current proposal dovetails with the other fish reproduction proposals being generated by the Washington State University/University of Idaho Center For Reproductive Biology. See Introduction to this Fish Reproduction Program for further details.

d. Project history (for ongoing projects) None

e. Proposal objectives

We hypothesize that **there is a direct relationship between ATP, and possibly PCr, content and motility and fertilizing ability of salmonid sperm and eggs**. To address this hypothesis, we will pursue the Specific Aims enumerated under Experimental Methods.

One objective of the proposed studies is to maintain our relationship with Dworshak National Fish Hatchery. We have a close relationship with the staff of the hatchery. They have shared their fish, facilities, and fertility data with us and we have shared our research data with them. (We have acknowledged their aid in several manuscripts: Bencic et al., In Press & Submitted.) Further, we share with them in advance the basic aspects of the studies we intend to perform.

The overall scientific objective is to elucidate the role(s) of ATP (and PCr) in the functional characteristics of salmonid gametes. These findings should then form the basis to develop mechanisms for improving the utilization of gamete samples particularly those from threatened or endangered populations.

The specific objectives of this FY2000 project are as follows:

1. Investigate whether the natural variation in sperm ATP, and presumably PCr, content correlates positively with variation in motility and fertilizing capability of sperm.

2. Investigate whether low sperm ATP and/or PCr content can be increased (and thus sperm quality increased) by specific manipulation in the laboratory.

3. Investigate the effects of cryopreservation on the relationship between ATP and/or PCr content and sperm motility and fertility.

Ultimately, the outcomes of these objectives focus on enhancing the quality of precious semen samples. They will be accompanied in FY2001-2004 by an analysis of salmonid egg quality and establishment of methods to maintain or enhance egg quality following short-term storage.

f. Methods: **General Methods and Procedures**:

Fish: Experiments will be conducted with both *Oncorhynchus mykiss* and *Oncorhynchus tshawytscha*; similarities in findings between these species suggest generalities among salmonids. The staff at Dworshak National Fish Hatchery (~80 mi from the Univ. of Idaho) have been very helpful in providing gametes from steelhead and fall run chinook. We are notified of their schedules in advance and are on site when adults are sacrificed. We always obtain semen within 30 min of collection (semen is kept on ice in the interim) and we obtain eggs upon opening of the females. Semen samples containing blood, urine, and/or fecal matter are not used. Upon obtaining the semen, antibiotics are added, samples are gassed (4-6X) and sealed, placed in larger bags which are gassed (4-6X) and sealed, and kept on ice for the return drive. Unless otherwise noted, eggs are pooled from several females and stored on ice for the return drive. At the Univ. of Idaho, gametes are kept at ~1^{o}C (unless otherwise noted).

It may not be practical to work on only Dworshak samples for two reasons. 1) While we have been able to obtain gametes from steelhead over 10 wk in spring, we only have a window of about 5 wk in the fall to obtain chinook gametes. These narrow temporal windows limit the data that can be obtained on either species per season. 2) Over the last 2 yr, the return of steelhead and chinook have been good and we have had access to gametes. If a future run is considered poor, gametes may not be shared with us. If/When samples are not available from Dworshak, some of the work will be conducted on gametes from commercially-raised rainbow trout. These gametes are shipped via Fed Ex from Mt. Lassen Trout Farm, Inc. (Red Bluff, CA) and are available year-round. Semen (under 100% O_2) and eggs are placed into ~1^{o}C refrigerator upon arrival and used fresh (or cryopreserved) within 24 hr.

Cryopreservation: Aliquots of semen are frozen in 300 mM glucose, 7.5% DMSO and 10% avian egg yolk. French straws (0.5 ml) filled with semen are frozen on dry ice for 15 min before being transferred into liquid N_2. Samples are thawed in a 4^{o}C water bath for 90 sec.

Sperm Viability: Sperm viability will be monitored to verify that decreases in semen ATP (and PCr) are not due to cell damage. A LIVE/DEAD sperm viability kit (Molecular Probes, Eugene, OR) is used with a Becton Dickinson FACScalibur flow cytometer to determine the fraction of live sperm. We have used this method successfully (Bencic et al., In Press; Submitted).

Motility and Fertility: Motility measurements will be *quantitative*. Motility will be assessed using an available WPI inverted phase microscopy (1 of 3 such systems in this department) and trials will be video-taped. Motility is based on the ratio of activated to nonactivated sperm following addition of 1-2µl milt to 100µl isotonic NaCl. Fertility studies are conducted by mixing sperm from individual males with eggs pooled from ≥3 females (unless otherwise specified) at a sperm to

egg ratio of 2 X 10^5 (Billard et al., 1974; Moccia & Munkittrick, 1987) This value may be adjusted down to enhance the resolution of sperm and egg treatment protocols. Eggs are incubated for 12 hr at 10°C then transferred to formalin-based Stockard's solution (Bencic et al., In Press) and later examined for cleavage (Ballard, 1973). Aliquots of eggs are also maintained with aged tap water in a Heath incubator (Heath Techna Inc., Tacoma WA;10-11°C; pH 8.5). Fertility is assayed on day 19 of incubation based on retinal pigmentation (an easily visible indicator of embryonic development).

ATP and PCr Assays: ATP levels are determined on neutralized perchloric acid (PCA) extracts of sperm with the luciferin-luciferase-based bioluminescence assay using an EG & G Berthold, Lumat LB 9507 luminometer. This method and instrument is very sensitive and reliable. We propose to determine PCr similarly, based on the creatine kinase-catalyzed, reversible reaction: PCr + ADP $\leftrightarrow$ Cr + ATP. To neutralized PCA extracts are added commercial creatine kinase and excess ADP and total ATP is then assayed via the luminometer. If this method is unsuccessful, we will use phosphorus NMR spectrometry to estimate concentrations by the method of Robitalle et al. (1987). To obtain and interpret these data for fee, we will use the NMR core facility operated by the Chemistry Dept, Univ. of Idaho. (If PCr appears to be a particularly important intracellular component, future studies will rely on HPLC analysis. HPLC analysis is much faster but initial costs are much higher than using available NMR spectrometers.)

Steelhead and chinook eggs are obtained within 10 min of stunning the female at Dworshak National Fish Hatchery. Upon collection, eggs plus an equal volume of ice cold PCA are scrambled with forceps, vortexed and placed onto dry ice. Egg+PCA mixtures are returned to the Univ. of Idaho and stored at -80°C until analysis. We are not aware of any reports of PCr in teleost eggs and it may not be present in measurable quantities. Nonetheless, it warrants examining. If several analyzes fail to demonstrate appreciable PCr levels (≥~10% of ATP levels), no further attempts at its quantitation be made.

Data Analysis: ANOVA will be followed by Newman-Keul's analysis; significance: $P \leq 0.05$.

Methods: Experimental Design

Specific Aim 1: Investigate whether the natural variation in sperm ATP, and presumably PCr, content correlates positively with variation in motility and fertilizing capability of sperm. Rationale: Using semen from both steelhead and chinook, we have noted a direct relationship between ATP content and motility and fertility after ATP levels are altered by incubation of samples at different O_2 tensions (prelim. data, Fig. 2). It is not clear, however, that the natural variation in ATP (and PCr) content of unmanipulated sperm correlates with motility and fertility. To address this Specific Aim, we will answer the following specific questions:

***Questions**: Does a direct relationship exist between ATP content and sperm motility and fertility for unmanipulated sperm from different males which contain different ATP levels? Does a positive correlation exist between PCr content and motility and fertility in unmanipulated sperm from different males?*

Steelhead and chinook eggs and semen samples will be collected at Dworshak Fish Hatchery and transferred on ice to the Univ. of Idaho. (Or, gametes from rainbow trout will be shipped from Mt. Lassen Trout Farm.) Eggs will be pooled from 3-6 females then aliquoted into groups of 200-250. Aliquots of semen will be mixed with ice-cold PCA immediately and kept on ice until analysis at UI for ATP (as well as for PCr); these represent T=0 values. Upon return to

UI, additional aliquots of semen will be analyzed to ensure that a reasonable range of ATP content exists in these samples. (Extra PCA extract will be stored at -80°C for subsequent PCr analysis.) Sperm samples, representing ~5 individual males, will also be examined for motility. To analyze for fertilizing capability, semen aliquots will be mixed with eggs at several dilutions and fertility checked at 24 hr by examining for cleavage and checked at ~ day 19 for retinal pigmentation. Subsequent PCr analysis will be used to test for a correlation between PCr content and sperm motility/fertility. Experiments will be replicated ≥3 times.

Anticipated Results: We predict that unmanipulated sperm samples containing relatively high ATP levels will be superior in motility and fertilizing ability to sperm samples containing relatively low ATP levels. Whether PCr will correlate to motility/fertility is not clear but is expected based on the findings of Robitaille et al. (1987) showing (via NMR) that PCr decreases, as does ATP, after sperm activation and motility.

Potential Problems and Corrective Actions: We have extensive experience measuring ATP levels in PCA extracts of sperm samples (Bencic et al., In Press; Submitted). To date, we have had technical problems with the enzymatic analysis of PCr; if we are unable to resolve these problems, we will resort to analysis by NMR spectroscopy.

Specific Aim 2: Investigate whether low sperm ATP and/or PCr content can be increased by specific manipulation in the laboratory. Rationale: Salmonid gametes maintain low levels of metabolism prior to fertilization. They consume endogenous nutrients and it is possible that limitations in endogenous nutrient supplies result in an inability of these cells to maintain ATP (and PCr) levels. Since it has been shown that exogenous nutrients can be taken up by these cells (Terner & Korsh, 1963a, b), we will examine the possibility that supplementing sperm with exogenous nutrients and 100% O_2 will reduce, eliminate or reverse the temporal decline in ATP previously noted with steelhead and chinook sperm samples. If this is possible, these results could have major implications for improving the quality of precious semen samples.

***Question**: Sperm depleted of ATP by transient anoxia (100% N_2) regenerate some of their initial ATP upon subsequent maintenance in seminal fluid under 100% O_2 (Fig 3, prelim studies). Therefore, can initial ATP, and possibly PCr, levels be regained after transient anoxia by maintenance with 100% O_2 plus nutrients?*

Semen samples will be analyzed for ATP (and possibly PCr) content and viability. Aliquots of semen will then be maintained at ~1°C for 8 hr in the absence of O_2 (100% N_2). Samples will be analyzed again for ATP (and possibly PCr) and viability, then split and maintained under N_2 or switched to 100% O_2 without or with additional organic nutrients (at 10 mM), especially pyruvate and acetate shown by Terner & Korsh (1963a,b) to support salmonid sperm metabolism. Samples will then be taken every 24 hr for ATP (and possibly PCr) analysis. Experiments will be replicated 3 times.

Anticipated Results: Based on our previous findings that sperm ATP levels rise appreciably when anoxia-maintained sperm are subsequently exposed to O_2, we believe that sperm have the capability of maintaining relatively high ATP levels in vitro. Whether this particular set of organic nutrients can lead to an elevation of ATP levels when sperm are also exposed to high levels of O_2 is not clear. If not, we will repeat these experiments with combinations of other organics such as glucose, glutamine, and fatty acids. If a particular combination is effective, the effectiveness of its individual components will be assessed.

Potential Problems and Corrective Actions: All technical steps (with the exception of PCr analysis) are straightforward and we anticipate no difficulties in obtaining the data.

***Question**: Can ATP levels be increased by incubation with O_2 and nutrients in samples not previously manipulated by transient exposure to anoxia?*

Untreated sperm as well as activated sperm will be incubated under 100% O_2 at 1°C with the nutrient-supplemented solution used in Specific Aim 2, Question A. (Sperm will be activated according to Bencic et al., In Press.) Samples will then be taken every 24 hr for ATP (and possibly PCr) analysis. Experiments will be replicated ≥3 times.

Anticipated Results: If successful in enhancing intracellular ATP levels in Specific Aim 2, Question A, as our preliminary data suggest, we predict success in enhancing unmanipulated samples in this experiment. Such enhancement could significantly improve the quality, and hence the value, of precious semen samples.

Potential Problems and Corrective Actions: All technical steps (with the exception of PCr analysis) are straightforward and we anticipate no difficulties in obtaining the data.

Specific Aim 3: Investigate the effects of cryopreservation on the relationship between sperm ATP and PCr content and sperm motility and fertility. Rationale: Since cryopreservation of sperm is an important practice in aquaculture and in the conservation of precious gametes, it is important to establish whether the proposed direct relationship between ATP and PCr content and fertilization success is maintained after such sperm storage.

***Question**: Does the positive relationship between sperm ATP and PCr content and motility and fertility persist in semen samples that have been cryopreserved?*

Aliquots of semen will be analyzed for intracellular ATP and PCr and motility and fertility before and after cryopreservation. Sperm will not be pooled among fish; samples will represent single males. Experiments will be replicated ≥3 times.

Anticipated Results: We predict that sperm ATP (and possibly PCr) content will correlate positively with sperm motility and fertility in stored by cryopreservation.

Potential Problems and Corrective Actions: Dr. Joseph Cloud is an expert in fish sperm cryopreservation and currently manages and maintains a germ plasm repository for threatened and endangered species of fishes at the Univ. of Idaho. Cryopreservation of semen samples will not be a problem.

Beyond FY2000:

Specific Aim 4: Investigate the variability and stability of intracellular ATP and PCr levels of eggs. Rationale: We do not know how stable egg ATP (and PCr) concentrations are during short-term storage. We also do not know whether maintenance/treatment protocols can be used to maintain or enhance their levels and thus, hopefully, maintain or enhance fertilizing success. However, the development of methods which elevate or prolong the quality of precious samples of eggs is highly desirable in salmonid recovery efforts.

***Question**: To what extent do egg ATP and PCr levels depend upon O_2 availability?*

Eggs will be collected as described and immediately transferred to plastic bags and flushed 4-6 times with humidified air, 100% O_2, or 100% N_2 (from gas cylinders kept at Dworshak). Groups of ~6 eggs per female will be PCA-extracted at 0 and 4 hr, and 1, 2, 4 and 6 d. A minimum of 4 values (representing different females) will be collected at each time point.

Anticipated Results: Based on the findings of carp eggs (Boulekbache et al., 1989), we expect that salmonid egg ATP levels will decline with time, especially under anoxia. As the Dworshak

staff have told us that ripe steelhead can be held several weeks longer than chinook before fertility drops precipitously, we predict a drop in egg ATP will be more pronounced in eggs of the latter. If a drop occurs quickly (~50% in 24 hr), experiments will be repeated at ~1^{o}C instead of 10^{o}C. Potential Problems and Corrective Actions: We anticipate no technical difficulties in obtaining the data. If egg viability becomes a concern during the duration of these experiments, we will add penicillin and streptomycin as we do to sperm samples.

Specific Aim 5: Investigate the relationship between egg ATP and PCr content and fertilization. Rationale: As we believe that there are many energy-dependent events taking place quickly at the time of fertilization, we predict that the ATP, and possibly PCr, content of the egg will control or influence fertilization success. If so, development of protocols which maintain, or perhaps enhance, intracellular ATP and/or PCr levels may be particularly valuable in increasing fertilization success after short-term egg storage.

***Question**: If egg ATP (and/or PCr) levels can be manipulated by ambient O_2, does a direct relationship exist between high ATP (and/or PCr) levels and fertilization?*

To eliminate the male as a variable, sperm from a single male will be aliquoted into straws and cryopreserved; those cryopreserved sperm samples will be used throughout this experiment. Eggs will be incubated for 24-48 hr at 10^{o}C under 0, 21, or 100% O_2. Several groups of ~6 eggs per female will be analyzed for ATP content; several other groups of eggs (~50) from the same lot will be mixed with the cryopreserved sperm. At 12 hr after mixing, the eggs will be fixed in Stockard's solution and examined for cleavage.
Anticipated Results: We suspect that maintenance in different O_2 tensions will result in a range of egg ATP levels. If so, we predict egg ATP content will correlate positively with fertilizing ability. Potential Problems and Corrective Actions: We will target a sperm-to-egg ratio yielding a fertilization rate of ~70% for controls to give sufficient resolution to yield interpretable results.

***Question**: Do eggs from different females with different mean egg ATP (and/or PCr) contents show difference in fertilization success?*

Eggs from ~6 females (to help ensure a range of egg ATP levels) will be returned to our lab and a group of ~6 eggs per female will be pooled and immediately analyzed for ATP. Groups of eggs (~50) from the same female will then be mixed with the cryopreserved sperm at several sperm-to-egg ratios then subsequently fixed in Stockard's solution. Eggs from the other females will be treated the same. At least 3 groups of ~6 females will be so analyzed.
Anticipated Results: If the quantity of stored ATP (and possibly PCr) in the egg is important in fertility, as we predict, we expect a direct relationship between fertility and initial ATP content. Potential Problems and Corrective Actions: We anticipate no difficulty in making the ATP measurements and analyzing fertility. This experiment assumes that a range of egg ATP values exists among females. However, if the variation among females is small, we may not be able to discern a direct relationship between stored ATP and fertility.

Specific Aim 6: If a direct relationship exists between ATP (and/or PCr) content and fertilization success, we will investigate whether specific maintenance conditions can stabilize or increase egg ATP content over short-term (few days) storage. Rationale: Only if there is a positive relationship between egg ATP (or PCr) content and fertilization success will this Specific Aim be pursued. If such a relationship is found, finding methods to maintain, or enhance egg ATP content would be very important in maintaining, or enhancing, egg quality of precious

samples during short-term storage. Although the ovulated teleost egg does not take up appreciable exogenous nutrients (Brooks et al., 1997), Terner (1968) showed salmonid eggs oxidize exogenous ^{14}C-acetate and ^{14}C-pyruvate to ^{14}C-CO_2. Also, Jensen and Alderdice (1984) showed higher salmonid egg viability at higher air-to-gamete ratios suggesting an importance of O_2 and oxidative metabolism in maintaining egg viability. These findings suggest that relatively low rates of nutrient uptake may supplement the normal (low) oxidation-based metabolism of the unfertilized egg and may extend the duration over which it can become fertilized.

***Question**: Does the maintenance of unfertilized eggs with organic nutrients (esp. acetate and pyruvate) under air or 100% O_2 influence any decrease in ATP content of stored eggs?*

Eggs (+ antibiotics) will be maintained for up to 6 days, under air or 100% O_2, at ~1 and 10^{o}C with and without 1mM acetate or pyruvate. At 0, 1, 2, 4, and 6, 10 d, eggs (from a single female) will be analyzed for ATP (and possibly PCr) content. This experiment will be replicated with eggs from an additional 3 females.

Anticipated Results: We expect these nutrients to be taken up by the unfertilized egg and metabolized. This may result in an enhanced concentration of ATP (and PCr) in the egg relative to controls. If not, the effectiveness of 3 and 10 mM concentrations will be examined. We do not know whether 21 or 100% O_2 will be the more effective gaseous environment.

Potential Problems and Corrective Actions: The experimental procedures are straightforward and we anticipate no difficulties in obtaining the data.

***Question**: Do the maintenance conditions found above result in enhanced fertilization success?*

This experiment will be conducted as described in Specific Aim 5, Question B.

Anticipated Results: We cannot predict what will happen but a positive outcome, an enhancement of fertilization, would have very significant implications on short-term storage of eggs and thus on salmonid recovery programs. This experiment is thus particularly important.

Potential Problems and Corrective Actions: The experimental procedures are straightforward and we anticipate no difficulties in obtaining the data.

g. Facilities and equipment Facilities and Equipment:

The laboratory space of the RLI, JGC, and JJN consists of 500 and 100 sq ft modules per investigator. The laboratories are maintained at 20^{o}C year round and contain a hood as well as line deionized water, compressed air and vacuum. RLI's office contains HP 4L laser printer and a Dell 120 mHz Pentium computer with internal fax/modem. RLI, JGC, and JJN each have a 100 sq ft office located in the same building, same floor as the laboratory modules. Three available 81 sq ft walk-in cold rooms (set at 2.5, 5, and 10^{o}C) are near the laboratory modules in the same building, same floor. The laboratory, office and cold rooms are housed in our research building which opened in 1986, has 56,000 sq ft and cost $11 million.

The RLI's laboratory has an EG & G Berthold, Lumat LB 9507 luminometer, a MG Industries 3-Channel Flo-Box gas mixer with Sierra Air/O_2, N_2 and CO_2 flow controllers, Servomex Oxygen Analyzer, a Model 905 Quantek Instr. O_2 and CO_2 analyzer, a Beckman J2-21 high speed centrifuge with JA-14, JA-20, JA-20.1, and JS-13 rotors, a Gilford Response spectrophotometer plus printer, Sartorius analytical balances, Rainin digital pipettors, Fisher microcentrifuges, 3WII Advanced Laboratory Wide-Range osmometer, Branson Cell Disruptor, an IEC Hematocrit centrifuge, and two refrigerators. The laboratories of JGC and JJN contain: Becton Dickinson FACScalibur flow cytometer, Beckman GPR centrifuge, EAC laminar flow

hood, Micron cryostat, Shimadzu UV spectrophotometer, pH meters, Westcor vapor pressure osmometer, Sartorius analytical balances, Nikon Diaphot inverted microscope, Wild and Olympus stereomicroscopes, Leitz dual micromanipulator, Olympus BH-2 microscope and camera, Beckman microfuge, 6X34 liter liquid nitrogen tanks, Cryo-Med controlled Rate Freezer, Gilson constant temperature water bath, Rainin digital pipettors, two eight-tray Health incubation units. The Department of Biological Sciences has three common equipment rooms that contain two Beckman ultracentrifuges, Savant Speed Vac, Virtis lyophilizer, ice machines and five -80^{o}C freezers. The department also maintains shared core histology, photography (darkroom), and molecular biology laboratories. The Department of Chemistry operates a core equipment laboratory and maintains 6 NMR spectrometers. NMR data will be generated for fee with an IBM NR300 NMR spectometer with the assistance of Dr. Gary Knerr, Instrument Supervisor, Dept. Chemistry.

An electronics shop, as part of the College of Engineering, is available as a support service. Full-time technicians in Electrical, Mechanical, and Chemical Engineering are also available.

Faculty members from the University of Idaho and Washington State University in Pullman (8 miles from Moscow) get together regularly as a Reproductive Biology Center. We hold frequent meetings and include graduate students from both campuses. This group, the research and library facilities, and other faculty of Washington State University greatly expand the intellectual and technical resources of researchers working at the University of Idaho.

h. Budget

Personnel:

Funds are requested for 3 months per year support ($15,000) for Rolf L. Ingermann, Associate Professor of Zoology, University of Idaho. RLI will devote 25% of the calendar year to this project. He will be responsible for overseeing the general experimental design, the execution of the studies, analysis of the data, and the writing of progress reports and manuscripts. He will also be directly involved in the research effort. Joseph G. Cloud, Professor of Zoology (1 month per year, $6,000), and James J. Nagler, Assistant Professor of Zoology (1 month per year for years 2-5, $5,000), University of Idaho, will provide expertise in sperm and egg physiology, methodologies, and data analysis. Support is requested for 1 technician ($34,000) and one graduate student ($15,000). These persons will be largely responsible for the collection trips to Dworshak Fish Hatchery, making of necessary solutions, and conducting of the necessary assays and motility and fertility assessments. The graduate student will also be actively involved in the analysis of data and the preparation manuscripts for publication. (The Department of Biological Sciences, Univ. Idaho pays the difference between in-state and out-of-state tuition for out-of-state graduate students admitted into the graduate program.) A part-time undergradate student ($6,000, $8.00/hr) will be responsible for the daily elimination of dead eggs from the fertility studies and for routine cleaning of laboratory glassware. Fringe benefits are set at 28.5% for faculty and the technician, 1% for graduate students and 1% for undergraduate students during the school year and 9% during the summer. Salaries and fringe benefits are increased by 5% per year.

Supplies, materials, nonexpendable property:

This category includes shipments of rainbow gametes from Mt. Lassen Trout Farm (shipped, eggs from 3 females and sperm from 3 males is $250), luminometer supplies & reagents (including a backup photomultiplier tube), dry ice & liquid nitrogen, miscellaneous reagents &

glassware and about $600 in computer software (probably: SigmaPlot & SigmaStat for data manipulation and statistical analysis). We request $600 for a video camera to record and quantitate motility studies. Current charges for use of the Becton Dickinson FACScalibur flow cytometer and the IBM NR300 NMR spectrometer are $60/hr (including the services of experts to ensure that the equipment is operating properly and help with data acquisition and interpretation). We anticipate a total of about 50 hr per instrument per year. These costs are increased by 5% per year.

Operations & maintenance:

We request $2,500 in graduate student tuition and fees. We will use a mini-truck from the College of Forest, Wildlife & Range Sciences, Univ. Idaho for an estimated 15 trips per year to Dworshak National Fish Hatchery, Ahsahka, Idaho, 80 miles from the University of Idaho. The charge for the vehicle is $8.00 per day plus $0.37/mile (gasoline is included in this price) and transportation costs are $1,000. Included are publication costs (eg, copying, photography, page charges, $1,000) as well equipment repair and maintenance support ($3,500) for the luminometer, high speed centrifuge, microcentrifuge, hematocrit centrifuge, spectrophotometer, pH meter, osmometers, oxygen analyzer, automatic pipets, calibration of electronic balances, etc. Requested funds are increased by 5% per year.

Capital acquisitions or improvements:

We request $2,500 to replace a 12 year old microcentrifuge and $2,500 to replace a 386 laboratory computer. These sums are only for use in the first year of the grant; zero funds are requested for years 2-5.

Travel:

The funds requested will be used by professional personnel to attend one national meeting per year. Examples of meetings include Experimental Biology, Society Study of Reproduction Meeting, American Fisheries Society, or Society for Integrative and Comparative Biology. Funds are increased by 5% per year.

Other:

The Administrative Core will integrate the activities of the Fish Reproductive Program and the Center for Reproductive Biology for administration of the grants, core laboratories, and activities such as seminars, workshops, and retreats. The integration of the different projects and research activities requires the Administrative Core support requested ($5,000; increased by 5% per year).

Section 9. Key Personnel

Rolf L. Ingermann

Dept. Biological Sciences, University of Idaho, Moscow, ID 83844-3051

Educational Background:

Ph.D., Biology, University of Oregon, Eugene, 1980
M.S., Biology, University of Oregon, 1974
B.A., Chemistry & Zoology, University of California, Los Angeles, 1972

Professional Experience:

Assoc. Prof. Zoology: 1992-present, University of Idaho, Moscow
Asst. Prof. Zoology: 1986-1992, University of Idaho

Asst. Prof. OB-GYN: 1984-1985, Oregon Health Sciences University, Portland
Postdoctoral Research Assoc.: 1980-1984, Oregon Health Sciences University

Role in Project:

RLI will devote 25% of the calendar year to this project. He will be responsible for overseeing the general experimental design, the execution of the studies (including collection of gametes from Dworshak National Fish Hatchery), analysis of the data, and the writing of progress reports and manuscripts. He will also be directly involved in the research effort.

Selected Publications:

(Bencic, DC, Krisfalusi, M, Cloud, JG, Ingermann, RL.[Submitted] Short-term storage of salmonid sperm in air versus oxygen. Progressive Fish Culturist)

Bencic, DC, Krisfalusi, M, Cloud, JG, Ingermann, RL. In Press. ATP levels of chinook salmon (*Oncorhynchus tshawytscha*) sperm following in vitro exposure to various oxygen tensions. Fish Physiology Biochemistry

Ingermann, RL, Bencic, DC, Herman, JK. 1997. Stability of nucleoside triphosphate levels in the red cell of the snake *Thamnophis elegans*. Journal of Experimental Biology 200: 1125-1131

Bencic, DC, Yates, TJ, Ingermann, RL. 1997. Ecto-ATPase activity of vertebrate blood cells. Physiological Zoology 70: 621-630

Ingermann, RL. 1992. Maternal-fetal oxygen transfer in lower vertebrates. American Zoologist 32: 322-330

Ingermann, RL. 1982. Physiological significance of Root effect hemoglobins in trout. Respiration Physiology, 49: 1-10

Joseph G. Cloud

Dept. Biological Sciences, University of Idaho, Moscow, Idaho 83844-3051

Educational Background:

Ph.D., Reproductive Physiology/Endocrinology, University of Wisconsin-Madison, 1974
M.Sc., Reproductive Physiology/Endocrinology, University of Wisconsin-Madison, 1968
B.Sc., Physiology/Pre-Veterinary Medicine, West Virginia University, 1966

Professional Experience:

Professor/Associate Professor/Assistant Professor of Zoology, University of Idaho, Moscow, 1977-present
Visiting Professor, University of Minnesota, 1983-1984
Assistant Professor, Johns Hopkins University, 1976-1977
Postdoctoral Fellow, Johns Hopkins University, 1974-1976

Role in Project:

JGC will devote 1 calendar month per year to this project. The overall project will rely on his extensive experience in quantifying sperm motility and will rely on his expertise in cryopreservation of sperm. He will be involved in overseeing the general experimental design, overseeing the analysis of the data, and will be involved in the writing of progress

reports and manuscripts.

Selected Publications:

Krisfalusi, M, Eroschenko, VP, Cloud, JG. 1998. Exposure of juvenile rainbow trout (*Oncorhynchus mykiss*) to methoxychlor results in a dose-dependent decrease in growth and survival but does not alter male sexual differentiation. Bulletin of Environmental Contamination and Toxicology 60: 659-666

Krisfalusi, M, Cloud, JG. 1996. Effects of exogenous estradiol-17ß on early growth and gonadal development of diploid and triploid female rainbow trout (*Oncorhynchus mykiss*). Developmental Genetics 19: 302-308

Greenlee, AR, Kersten, CA, Cloud, JG. 1995. Effects of triploidy on rainbow trout myogenesis in vitro. Journal of Fish Biology 46: 381-388

Thorgaard, GH, Cloud, JG. 1993. Reconstitution of genetic strains of salmonids using biotechnical approaches. In: "Genetic Conservation of Salmonid Fishes" (J.G. Cloud & G.H. Thorgaard, eds.) Plenum Press, New York, pp. 189-196

Nilsson, EE, Cloud, JG. 1992. Rainbow trout chimeras produced by injection of blastomeres into recipient blastulae. Proceedings of the National Academy of Science USA 89: 9425-9428

James J. Nagler

Dept. Biological Sciences, University of Idaho, Moscow, Idaho 83844-3051

Educational Background:

Ph.D., Fish Reproductive Physiology, Memorial University of Newfoundland, Canada, 1991

M.Sc., Fish Reproductive Toxicology, Concordia University, Canada, 1985

B.Sc., Fish and Wildlife Biology, University of Guelph, Canada, 1983.

Professional Experience:

Assistant Professor of Zoology, 1996-present, University of Idaho, Moscow

Role in Project:

JNN will not be directly involved in this project for FY2000. He will devote 1 calendar month per year to this project thereafter. The portions of the project which focus on salmonid egg analysis and enhancement will rely on his interest and expertise in salmonid egg quality assessment. After the initial year, he will be involved in overseeing the general experimental design, overseeing the analysis of the data, and will be involved in the writing of progress reports and manuscripts.

Selected Publications:

Nagler, JJ, Cyr, DG. 1997. Exposure of male American plaice (*Hippoglosoides platessoides*) to contaminated marine sediments decreases the hatching success of their progeny. Environmental Toxicology and Chemistry 16: 1733-1738

Nagler, JJ, Scott, AP, Tyler, C, Sumpter, JP. 1996. Gonadotropins I and II do not stimulate the *in vitro* secretion of 17a,20ß-dihydroxy-4-pregnen-3-one 20-sulphate by rainbow trout gonads during final sexual maturation. Fish Physiology and

Biochemistry 15: 149-156.
Tyler, CR, Pottinger, TG, Santos, E, Sumpter, JP, Price, S-A., Brooks, S, Nagler, JJ. 1996. Mechanisms controlling egg size and number in the rainbow trout *Oncorhynchus mykiss*. Biology of Reproduction 54: 8-15.
Nagler, JJ, Tyler, CR, and Sumpter, JP. 1994. Ovarian follicles of rainbow trout (*Oncorhynchus mykiss*) cultured within lamellae survive well, and sequester and process vitellogenin. Journal of Experimental Zoology 269: 45-52.
Nagler, JJ, Idler, DR, So, YP. 1992. A comparison of tritiated vitellogenins prepared by in vivo and in vitro techniques for studies of ovarian uptake in fish. Journal of Experimental Zoology 264: 107-112.

Section 10. Information/technology transfer

◆ Findings which have impact on hatchery practices will be shared with the staff at Dworshak National Fish Hatchery as well as with personnel at other hatchery facilities.

◆ Findings will be presented regularly at scientific conferences. Conferences attended will be hosted by the Society Study of Reproduction Meeting, American Fisheries Society, Experimental Biology, and/or Society for Integrative and Comparative Biology.

◆ Summary of major findings will be presented as progress reports annually to BPA.

◆ Most importantly, findings will be published in peer-reviewed, scientific journals with global distributions.

Congratulations!